life-style risk factors relating to the fact that cannabis consumption is frequently associated with tobacco use and to some festive habits in young males (3). The higher prevalence of intracranial arterial stenosis as a cause of IS in CU in our series is in accordance with the potential involved mechanisms of stroke in CU represented by reversible cerebral vasoconstriction, and impairment of cerebral mitochondrial respiration induced by tetrahydrocannabinol (4,5). The reversibility of vasoconstriction was also described in peripheral arteritis associated with cannabis use (3). Our data demonstrate that a favorable functional capacity is common in young patients suffering from stroke independently of cannabis use that is likely due to age-related enhanced brain plasticity. However, in the whole series, 18% of patients retained significant disability, along with 5 deaths.

Fighting stroke must remain a priority, including in young adults. The first step may be to inform the public regarding the potential occurrence of stroke associated with cannabis use and other life-style risk factors (3), particularly nowadays when cannabis use is encouraged by new legislations worldwide.

ACKNOWLEDGMENTS The authors are grateful to François Piquard, PhD, and Rodrigue Galani, PhD, for performing the statistical analysis.

*Valérie Wolff, MD, PhD
Ielyzaveta Zinchenko, MD
Véronique Quenardelle, MD
Olivier Rouyer, MD, PhD
Bernard Geny, MD, PhD

*Unité neuro-vasculaire, Service de Neurologie
Hôpitaux Universitaires de Strasbourg
1, avenue Molière
67 098 Strasbourg
France
E-mail: valerie.wolff@chru-strasbourg.fr

http://dx.doi.org/10.1016/j.jacc.2015.08.867

Please note: The authors have reported that they have no relationships relevant to the contents of this paper to disclose.

REFERENCES

1. Maaijwee NA, Rutten-Jacobs LC, Schaapsmeerders P, et al. Ischaemic stroke in young adults: risk factors and long-term consequences. Nat Rev Neurol 2014;10:315–25.

2. Bejot Y, Daubail B, Jacquin A, et al. Trends in the incidence of ischaemic stroke in young adults between 1985 and 2011: the Dijon Stroke Registry. J Neurol Neurosurg Psychiatry 2014;85:509–13.

3. Thomas G, Kloner RA, Reskalla S. Adverse cardiovascular, cerebrovascular, and peripheral vascular effects of marijuana inhalation: what cardiologists need to know? Am J Cardiol 2014;113:187–90.

4. Wolff V, Armspach JP, Beaujeux R, et al. High frequency of intracranial arterial stenosis and cannabis use in ischaemic stroke in the young. Cerebrovasc Dis 2014;34:438–43.

5. Wolff V, Rouyer O, Geny B. Adverse health effects of marijuana use. N Engl J Med 2014;371:878–9.

Association of *APOC3* Loss-of-Function Mutations With Plasma Lipids and Subclinical Atherosclerosis

The Multi-Ethnic BioImage Study

In 2008, Pollin et al. (1) identified 1 mechanism of lowering triglyceride-rich lipoproteins among the Lancaster Amish, loss of apolipoprotein C-III (*APOC3*) gene function, which was associated with reduced subclinical atherosclerosis (i.e., coronary arterial calcification on cardiac computed tomography). We recently extended these observations to show that *APOC3* loss-of-function mutations also reduce risk for clinical atherosclerotic cardiovascular disease (ASCVD) (2,3). Here, we address 2 questions related to mutations that reduce APOC3 function: 1) do these mutations also associate with lower plasma low-density lipoprotein cholesterol (LDL-C); and 2) do these mutations reduce subclinical atherosclerosis in the general population, particularly in individuals with ancestry outside of Europe?

We studied 6,699 individuals of European, African, Asian, and Hispanic ancestries from the BioImage Study (BioImage Study: A Clinical Study of Burden of Atherosclerotic Disease in an At-Risk Population, NCT00738725), a prospective, observational study aimed at characterizing subclinical atherosclerosis in U.S. adults (55 to 80 years old) at risk for clinical ASCVD (4). We genotyped 4 *APOC3* (NM_000040.1) loss-of-function mutations (IVS2+1G→A, A43T, R19X, IVS3+1G→T) using the Illumina HumanExome BeadChip Array v1.1 (Illumina, San Diego, California). Written informed consent was obtained from all study participants according to a protocol approved by the Western Institutional Review Board, Olympia, Washington. Fasting blood lipids were measured at the baseline examination. Blood lipids were adjusted for the presence of statin medications to reflect the observation that statins, on average, reduce total cholesterol by 20% and LDL-C by 30% (5). Noninvasive assessments for subclinical atherosclerosis (coronary arterial calcification [CAC], carotid plaque, and carotid intima media thickness [CIMT]) were performed at the baseline examination on a mobile imaging facility as previously described (4).

A total of 6,395 subjects passed all quality-control measures. Variant calling was performed using

TABLE 1 Association of *APOC3* Loss-of-Function Mutation Carrier Status With Blood Lipid Levels and Subclinical Atherosclerosis

	Noncarriers (n = 6,331)	Carriers (n = 64)	Effect Estimate	95% CI	p Value
Blood lipids					
Triglycerides, mg/dl*	166.1 ± 96.2	91.2 ± 44.1	−43.7%	−36.6 to −49.9	1.83×10^{-21}
HDL cholesterol, mg/dl	55.6 ± 15.2	66.6 ± 15.0	+11.1	7.6 to 14.6	3.55×10^{-10}
LDL cholesterol, mg/dl	130.3 ± 36.4	131.6 ± 35.8	+1.5	−7.4 to 10.3	0.75
Subclinical atherosclerosis					
Coronary arterial calcification, Agatston units†	46.0 (0.0 to 245.0)	29.0 (0.0 to 227.5)	−27.9	−51.1 to −4.7	0.019
Carotid plaque, mm^2†	183.8 (0.0 to 555.9)	112.8 (0.0 to 367.2)	−8.7	−73.5 to 56.0	0.79
Carotid intima media thickness, mm*	0.76 ± 0.16	0.74 ± 0.13	−1.7%	−6.3 to 3.0	0.47

Values are represented as effect estimates (95% confidence intervals) between *APOC3* loss-of-function mutation carriers and noncarriers after adjustment for covariates. Covariates used were age, sex, ancestry, and principal components of ancestry. *Effect estimates for triglycerides and carotid intima media thickness were derived from natural log transformation and are expressed as percent difference in carriers compared with noncarriers. †Effect estimates for coronary arterial calcification and carotid plaque derived from median quantile regression and represent differences in medians. Therefore, summary statistics of the distributions in noncarriers and carriers are represented as median (interquartile range).

CI = confidence interval; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

GenCall (Illumina) and zCall (5). Sixty-four heterozygous carriers of *APOC3* loss-of-function mutations (25 IVS2+1G→A, 25 A43T, 13 R19X, 1 IVS3+1G→T; combined minor allele frequency of 0.5%) were identified. Principal components were derived from a set of high-quality, independent variants on the genotyping array using Eigenstrat as has previously been done (3,5). To minimize confounding from systematic differences in allele frequencies by trait, we reduced the observed genetic variation to the top eigenvectors derived from the sample covariance matrix. To test the association of *APOC3* loss-of-function mutation with an outcome, linear regression was used for triglycerides, LDL-C, and high-density lipoprotein cholesterol (HDL-C), and CIMT; triglycerides and CIMT were natural log-transformed. And given the bimodal, skewed distributions of CAC (primary outcome) and carotid plaque, median quantile regression was used for these 2 variables. Age, sex, ethnicity, and principal components of ancestry were used as covariates in all analyses. Given a 2-sided alpha threshold of 0.05, we have >80% power to detect an effect size of 0.16% of variance explained for analyzed traits.

Among carriers and noncarriers of the *APOC3* loss-of-function mutations, there were no significant differences in age, sex, hypertension, diabetes mellitus, body mass index, current smoking, aspirin use, or statin use. There were no significant differences in proportions of carriers amongst each ethnicity group ($p > 0.20$). We replicated the finding that *APOC3* loss-of-function mutations were associated with reduced triglycerides (−43.7%; $p = 1.83 \times 10^{-21}$) and increased HDL-C (11.1 mg/dl; $p = 3.55 \times 10^{-10}$), with a larger standardized effect on triglycerides compared with HDL-C (−1.17 SD vs. +0.73 SD). When accounting for statin treatment, carriers did not have different LDL-C concentrations compared with noncarriers ($p = 0.75$).

APOC3 loss-of-function mutations were associated with decreased median CAC score (−27.9 U; 95% confidence interval [CI]: −51.08 to −4.67; $p = 0.019$) across all phenotyped participants ($n = 5,631$); this effect was consistent in those of European ancestry (−27.5 U; 95% CI: −67.1 to 12.1) and of non-European ancestry (−5.62 U; 95% CI: −39.2 to 27.9). Neither carotid plaque ($p = 0.79$) nor CIMT ($p = 0.47$) ($n = 5,746$) differed between carriers and noncarriers (Table 1).

In a multiethnic study of U.S. adults, *APOC3* loss-of-function mutation carriers had reduced plasma triglycerides, higher HDL-C, and a decreased burden of coronary arterial calcification. These data support the notion that *APOC3* deficiency reduces coronary atherosclerosis in the general population. Whether pharmacological inhibition of *APOC3* will reduce ASCVD risk remains to be tested.

Pradeep Natarajan, MD, MMSc
Puja Kohli, MD
Usman Baber, MD
Khanh-Dung H. Nguyen, PhD
Samantha Sartori, PhD
Dermot F. Reilly, PhD
Roxana Mehran, MD
Pieter Muntendam, PhD
Valentin Fuster, MD, PhD
Daniel J. Rader, MD
***Sekar Kathiresan, MD**

*Center for Human Genetic Research and
Cardiovascular Research Center
Massachusetts General Hospital
185 Cambridge Street, CPZN 5.252
Boston, Massachusetts 02114
E-mail: skathiresan1@mgh.harvard.edu

http://dx.doi.org/10.1016/j.jacc.2015.08.866

(BioImage Study: A Clinical Study of Burden of Atherosclerotic Disease in an At-Risk Population [BioImage Study]; NCT00738725)

Please note: This work was supported by a grant from Harvard Medical School (John S. LaDue Memorial Fellowship in Cardiology, to Dr. Natarajan), grants from the NHLBI (T32HL116275, to Dr. Kohli; R01HL107816, to Dr. Kathiresan), and a grant from the Donovan Family Foundation, an investigator-initiated research grant from Merck & Co. and a grant from Fondation Leducq (all to Dr. Kathiresan). Dr. Nguyen is an employee of Merck & Co. Dr. Reilly is an employee of and holds stock in Merck & Co. Dr. Mehran has received grant support from BG Medicine, Eli Lilly and Company, AstraZeneca, The Medicines Company, and BMS/Sanofi; is a consultant for AstraZeneca, Bayer, CSL Behring, Janssen Pharmaceuticals, Merck & Co., Osprey Medical, and Watermark Research Partners; and is on scientific advisory boards of Abbott Laboratories, Boston Scientific Corporation, Covidien, Janssen Pharmaceuticals, The Medicines Company, and Sanofi. Dr. Muntendam is an employee of and shareholder in scPharmaceuticals. Dr. Kathiresan has received research grants from Regeneron, Bayer, Aegerion; is on the scientific advisory board for Catabasis, Regeneron Genetics Center, Merck, Celera; holds equity in Catabasis, San Therapuetics; and is a consultant for Novartis, Aegerion, Bristol-Myers Squibb, Sanofi, AstraZeneca, Alnylam, Eli Lilly, Leerink Partners, and Merck. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose. Michael Miller, MD, served as Guest Editor for this paper. The High-Risk Plaque (HRP) Initiative encompassing the BioImage Study is a pre-competitive industry collaboration funded by Abbott, Abbvie, AstraZeneca, BG Medicine, Merck & Co., Philips, and Takeda. HRP Joint Steering Committee: Pieter Muntendam, MD (BG Medicine); Aram Adourian (BG Medicine); Michael Klimas, PhD (Merck); Joel Raichlen, MD (AstraZeneca); Oliver Steinbach (Philips); James Beckett (Philips); Ramon Espaillot (Abbvie); Michael Jarvis (Abbvie); and Tomoyuki Nishimoto (Takeda). The sponsor had no role in the study design; in the collection, analysis, and interpretation of the data; in the writing of this report; or in the decision to submit the paper for publication

REFERENCES

1. Pollin TI, Damcott CM, Shen H, et al. A null mutation in human APOC3 confers a favorable plasma lipid profile and apparent cardioprotection. Science 2008;322:1702–5.

2. Jorgensen AB, Frikke-Schmidt R, Nordestgaard BG, et al. Loss-of-function mutations in APOC3 and risk of ischemic vascular disease. N Engl J Med 2014;371:32–41.

3. Crosby J, Peloso GM, Auer PL, et al. Loss-of-function mutations in APOC3, triglycerides, and coronary disease. N Engl J Med 2014;371:22–31.

4. Baber U, Mehran R, Sartori S, et al. Prevalence, impact, and predictive value of detecting subclinical coronary and carotid atherosclerosis in asymptomatic adults: the BioImage Study. J Am Coll Cardiol 2015;65:1065–74.

5. Peloso GM, Auer PL, Bis JC, et al. Association of low-frequency and rare coding-sequence variants with blood lipids and coronary heart disease in 56,000 whites and blacks. Am J Hum Genet 2014;94:223–32.

Analysis of Dual Antiplatelet Therapy

In a post-hoc analysis, Yeh et al. (1) provide evidence that dual-antiplatelet therapy with thienopyridine and aspirin, for 30 (vs. 12) months after coronary stenting, had beneficial effects regardless of whether patients had acute myocardial infarction (MI) or less acute presentations. In particular, stent thrombosis was reduced—although bleeding was increased—similarly in both groups. Clinicians should, however, consider various elements of the study design when interpreting results and using the findings in clinical practice.

Importantly, the study classified patients with unstable angina as having less acute ("no MI") presentations. This subset accounted for nearly one-fourth (22.6%) of the 8,072 patients without MI, or roughly 1,800 patients—representing more than one-half of the 3,576 patients with MI. Combining unstable angina patients within the non-MI group is of more utility in allowing for conclusions to be drawn on the MI group, given that the non-MI group represents a population of patients with a wide range of risk of subsequent events—and it is unclear whether the beneficial effects of prolonged treatment was driven by those at higher risk within this group. Perhaps a sensitivity analysis could be done, excluding patients with unstable angina from the non-MI group, to see if the overall results are affected.

From a more general perspective, the reader may not immediately recognize that the current analyses represent a post-hoc subgroup analysis of a previously completed randomized trial, with randomization 12 months after initial enrollment. We would defend the authors from reflexive criticisms that such analyses are always problematic, but we also note that whether the findings are valid, or due to chance or bias, is always a consideration. For example, lacking a formal power calculation linked to antecedent MI status, the discordant findings of similarly reduced stent thrombosis without an interaction, compared to differential reduction of major adverse cardiovascular and cerebrovascular events with an interaction, have uncertain implications.

Finally, as a specific methodological issue, the data in Figure 1 in the paper by Yeh et al. (1) indicate that (25,682 total minus 5,844 not eligible equals) 19,838 patients were eligible for randomization, but 8,190 were not randomized at 12 months for the reasons stated. Given that this group represents (8,190 divided by 19,838 equals) 41.3% of eligible patients, it would be useful to know whether pertinent baseline characteristics (including the distribution of patients in MI and non-MI groups) differ between analyzed and excluded patients, for better understanding the overall results.